

## CLAIMS

1. Method for the preparation of a strain of evolved micro-organisms for the production of 1,2-propanediol by the metabolism of a simple carbon source,  
5 which method comprises the growth, under selection pressure in an appropriate growth medium containing a simple carbon source, of an initial bacterial strain that has undergone deletion of the gene *tpiA* and the deletion of at least one gene involved in the conversion of methylglyoxal (propanal) into lactate, in order to cause to evolve, in said strain, one or  
10 more genes involved in the biosynthesis pathway from DHAP to methylglyoxal and then to 1,2-propanediol towards evolved genes that possess an improved "1,2-propanediol synthase" activity, which resulting evolved strain or strains of micro-organisms that possess an improved "1,2-propanediol synthase" activity are then selected and isolated.
- 15 2. Method according to Claim 1, characterised in that the gene involved in the conversion of methylglyoxal into lactate is *gloA*, *aldA* or *aldB*.
3. Method according to either of Claims 1 or 2, characterised in that the initial strain has undergone the deletion of the genes *gloA*, *aldA*, *aldB* and *tpiA*.
4. Method according to any of Claims 1 to 3, characterised in that the initial  
20 strain has also undergone the deletion of the genes *ldhA*, *pflA*, *pflB*, *adhE* and *edd*.
5. Method according to any of Claims 1 to 4, characterised in that the initial strain also contains at least one gene coding for an enzyme that favours the metabolism of pyruvate to acetate.
- 25 6. Method according to Claim 5, characterised in that the enzyme that favours the metabolism of pyruvate into acetate has low sensitivity to inhibition by NADH.
7. Method according to either of Claims 5 or 6, characterised in that the said enzyme favours the metabolism of pyruvate towards the production of  
30 acetyl-CoA and NADH.
8. Method according to Claim 7, characterised in that the enzyme is a pyruvate dehydrogenase complex.

9. Method according to any of Claims 6 to 8, characterised in that the enzyme that favours the metabolism of pyruvate into acetate is an endogenous enzyme.
- 5 10. Method according to any of Claims 1 to 9, characterised in that one or more heterologous genes coding for one or more enzymes involved in the conversion of acetyl-CoA and acetate into acetone are introduced into the evolved strain.
- 10 11. Method according to Claim 10, characterised in that the heterologous gene or genes coding for one or more enzymes involved in the conversion of acetyl-CoA and acetate are from *C. acetobutylicum*.
12. Method according to either of Claims 10 or 11, characterised in that an evolved modified strain obtained according to either of Claims 10 or 11 is grown under selection pressure in an appropriate growth medium containing a simple carbon source in order to cause, in said evolved modified strain, the evolution of one or more genes involved in the conversion of acetyl-CoA and acetate to acetone towards an improved "acetone synthase" activity. The second generation of resulting evolved micro-organisms that possess an improved "1,2-propanediol synthase" activity and an improved "acetone synthase" activity are then selected and isolated.
- 15 20 13. Method according to any of the preceding claims, characterised in that the strain is a bacterium, a yeast or a fungus.
14. Method according to Claim 13, characterised in that the strain is a strain of *Escherichia*, in particular *E. coli*, and *Corynebacterium*, in particular *C. glutamicum*.
- 25 15. Initial strain as defined according to any of Claims 1 to 9.
16. Evolved strain that can be obtained by the method according to any of Claims 1 to 14.
- 30 17. Strain according to Claim 16, in which the gene *lpd* has a point mutation whereby alanine 55 is replaced by valine.

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18. Method of preparation of 1,2-propanediol in which an evolved strain is grown according to either of Claims 16 or 17 in an appropriate growth medium containing a simple carbon source, and in which the 1,2-propanediol produced is recovered.
  19. Method according to Claim 18, characterised in that 1,2-propanediol and acetone are recovered.
  20. Method according to either of Claims 18 or 19, characterised in that the 1,2-propanediol and/or acetone are purified.